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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Georgia L. Helmer
Group	:	1638
Applicants	:	Michael Wassenegger, et al.
Serial No.	:	09/782,874
Filed	:	February 8, 2001
For	:	NUCLEIC ACID MOLECULES ENCODING POLYPEPTIDES HAVING THE ENZYMATIC ACTIVITY OF AN RNA-DIRECTED RNA POLYMERASE (RdRP)

Hon. Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MICHAEL WASSENEGGER UNDER 37 C.F.R. § 1.132

I, Michael Wassenegger, a citizen of Germany, residing at Schellingstrasse 22, 80799 Munich, Germany hereby declare that:

1. I received a diploma in biology in 1984 and a doctoral degree in biochemistry in 1988 from the University of Cologne/Max-Planck-Institute for Plant Breeding Research. I have published twenty scientific articles, and have received two patents. My curriculum vitae is attached as Exhibit 1.

2. I have studied gene silencing in plants since 1994. I have been employed by the Fraunhofer Institute for Molecular Biology and Applied Ecology since 1999 as head of the Epigenetics department.

3. I am familiar with the November 20, 2002 Office action in the above-identified application. I understand it is the Examiner's view that the claims contain subject matter which was not described in the specification in such a way as

to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention at the time the application was filed. Specifically, in the Examiner's view:

> [t]he claims are drawn to sequences that are at least 60% identical to SEQ ID NO: 1 that encodes a protein at least 60% identical to SEQ ID NO: 2 or to conservative variants thereof that have RdRP activity. However, the specification does not disclose what structural features would be conserved in the claimed sequences that would result in the claimed enzyme activity. Applicants are claiming a genus of sequences, yet there is no description of the structural features that define the genus.

See Office Action, page 7.

4. In addition, I understand that the Examiner considers the disclosure enabling only for claims directed to a nucleic acid molecule that comprises a nucleic acid sequence that is SEQ ID NO: 1, or that encodes SEQ ID NO: 2, where the nucleic acid encodes a protein that has RdRP activity. See Office Action, page 8. In the view of the Examiner, the disclosure does not provide enablement for:

> sequences that are at least 60% identical to SEQ ID NO: 1, that encodes a protein at least 60% identical to SEQ ID NO: 2 or to conservative variants thereof that have RdRP enzymatic activity . . . [or for] a nucleic acid of SEQ ID NO: 1 or a nucleic acid encoding SEQ ID NO: 2, where that nucleic acid (presence/transcription/expression) causes a reduction in synthesis of RdRP.

See Office Action, page 8.

5. I make this declaration to demonstrate that the specification adequately describes the claimed transgenic plant cells and transgenic plants comprising the nucleic acid molecule of the invention. I further make this declaration to demonstrate that sequences that are at least 80% identical to SEQ ID NO: 1 or that encode a protein at least 80% identical to SEQ ID NO: 2, as well as degenerate

sequences thereof, encode a protein having RdRP activity.[1] Finally, I make this declaration to demonstrate that one of ordinary skill in the art, following the teachings of the specification could produce transgenic plants and plant cells containing the nucleic acid molecules of the invention and would reasonably expect that such integrated constructs would, through transcription and/or expression of the nucleic acid molecule, reduce RdRP activity in the cell. Specifically, I describe experiments demonstrating the production of transgenic tobacco plants with integrated nucleic acid molecules encoding RdRP in antisense orientation that show reduced RdRP activity.

6. The specification describes a Southern blot analysis of the *Hind*III-restricted genomic DNA of two different tomato species, potato and tobacco. See Fig. 2 of the specification. The Southern blot, using a ^{32}P-labeled tomato RdRP DNA probe, reveals that the RdRP gene is present in each of the four genomes. Further, the Southern blot analysis provides evidence that the inventors had possession of a nucleic acid molecule encoding RdRP in various plant species. As discussed in ¶7 below, the nucleotide sequence comparison between the RdRP coding region of *Nicotiana tabacum* to that of SEQ ID NO: 1 shows a 89.9% sequence identity while the amino acid sequence comparison between *Nicotiana tabacum* and SEQ ID NO: 2 shows a sequence identity of 85.8%. For these reasons, I believe that the written description in the specification sufficiently conveys the structural and physical features of the claimed transgenic plant cells and plants comprising nucleic acid molecules that have at least 80% sequence identity to SEQ ID NO: 1 or that encode a protein at least 80% identical to SEQ ID NO: 2 that result in the claimed RdRP activity. Therefore, it is my belief, based on the evidence, that the specification

[1] Three RdRP molecules have been identified since our initial identification of RdRP in plants. The originally identified RdRP molecule is now referred to as RdRP[1]. However, in this declaration, I refer to it as it was originally described.

reasonably conveys to one of ordinary skill in the art that the inventor was in possession of the claimed genus at the time the application was filed.

7. The specification enables nucleic acid molecules having at least 80% sequence identity to SEQ ID NO: 1 or to a nucleic acid molecule encoding SEQ ID NO: 2, as well as to degenerate sequences thereof. Nucleic acid molecules encoding RdRP from *Nicotiana tabacum* and *Arabidopsis thaliana* species have been isolated and sequenced. See Exhibit 2. Comparisons of these sequences to SEQ ID NO: 1 and SEQ ID NO: 2 are attached as Exhibits 3-5. The RdRP amino acid sequences of *Nicotiana tabacum* and *Arabidopsis thaliana* exhibit a sequence identity to SEQ ID NO: 2 of 85.8% and 61.9%, respectively. See Exhibit 3. The RdRP coding region of *Nicotiana tabacum* and *Arabidopsis thaliana* exhibit a nucleotide sequence identity to the coding region of SEQ ID NO: 1 of 89.9% and 63.2%. See Exhibits 4 and 5. Xie et al. (PNAS, 99:6516-6521, 2001; hereafter "Xie") demonstrates that the nucleic acid molecule encoding the *N. tabacum* RdRP (NtRdRP) has RdRP activity. See, e.g., pages 6517-6519 of Xie. Further, one having skill in the art would reasonably expect that these *Arabidopsis* nucleic acid molecules would also encode polypeptides with RdRP activity. Thus, the comparison of the nucleic acid molecules of this invention with corresponding sequences from *Nicotiana tabacum* and *Arabidopsis thaliana* demonstrate that nucleic acid molecules exhibiting a sequence identity of approximately 80% or more to the nucleic acid molecules of this invention encode a polypeptide with RdRP activity.

8. In addition, the specification teaches one of ordinary skill in the art to identify and isolate nucleic acid molecules that exhibit at least 80% sequence identity to the described nucleic acid sequences that encode a protein having RdRP activity. See page 7, line 28 to page 8, line 23 of the specification. For example, the specification also teaches and exemplifies an assay for determining whether a protein

encoded by a nucleic acid molecule of the invention has RdRP activity. See page 31, line 19-31 of the specification. Thus, given the teachings in the specification as filed, I believe that others skilled in the art could make and use nucleic acid molecules that exhibit a sequence identity of at least 80% to SEQ ID NO: 1 or that encode a protein that is at least 80% identical to SEQ ID NO: 2 without undue experimentation.

9. The specification provides enablement where the presence, expression, or transcription of a nucleic acid having at least 80% sequence identity to SEQ ID NO: 1 or a nucleic acid molecule that is at least 80% identical to a nucleic acid molecule encoding SEQ ID NO: 2 causes a reduction in RdRP activity. The specification discloses that expression of a nucleic acid molecule of the invention in a plant cell would reduce the synthesis of a polypeptide having RdRP activity due to an antisense or co-suppression effect. See page 22, lines 6-12 of the specification. The specification further teaches how to achieve such an antisense or co-suppression effect with the nucleic acids of the invention. Specifically, the specification discloses that the nucleic acid molecules of the invention are preferably linked to regulatory elements ensuring transcription in plant cells, and describes specific regulatory elements that could be used. The specification teaches that the nucleic acid molecule may be of homologous origin with respect to the plant species used for transformation or may be a heterologous nucleic acid molecule, preferably with homology of at least 80%. See page 23, lines 17-25 of the specification. The specification also teaches methods for transforming monocotyledonous and dicotyledonous plant cells with antisense nucleic acid constructs, and methods for identifying transgenic plant cells having the desired characteristics. The specification also teaches regeneration of transgenic plants from the transgenic plant cells. See page 23, line 27 to page 25, line 13 of the specification.

10. Xie discloses the suppression of inducible RdRP activity in transgenic tobacco plants into which an antisense construct of the homologous tobacco RdRP gene (NtRdRP) had been introduced. Specifically, Xie cloned a 3.8 kb NtRdRP cDNA fragment into the plant transformation vector pCK8 in antisense orientation under the regulation of the cauliflower mosaic virus (CaMV) 35S promoter. See Xie, page 6517, right column, lines 4-10. The instant specification teaches the use of expression vectors and various promoters including the 35S promoter of CaMV. See page 24, lines 3-5 and page 10, lines 4-15 of the specification. Xie introduced the NtRdRP antisense construct into tobacco by *Agrobacterium*-mediated transformation. See Xie, page 6517, right column, lines 4-10. The instant specification teaches that the foreign DNA of the invention may be introduced into plants by *Agrobacterium*-mediated transformation. See page 24, line 28 to page 25, line 2 of the specification.

11. Xie demonstrated that salicylic acid (SA) or tobacco mosaic virus (TMV) treatment induced RdRP activity in wild type plants. See Xie, Fig. 3. In contrast, transgenic tobacco plants containing the antisense RdRP construct exhibited drastically reduced RdRP activity after treatment with SA or TMV (see Xie, Figs. 3A and 3B) and contained no detectable NtRdRP transcripts after viral infection (see Xie, Figs. 3C and 3D). Xie also showed that the antisense transgenic plants accumulated higher levels of viral RNAs (see Xie, Fig. 4) and developed more severe symptoms after infection by TMV (see Fig 5) or potato virus X (see Xie, Fig 6). Thus, Xie demonstrates that expression of an RdRP nucleic acid molecule in antisense orientation reduces RdRP activity.

12. Following the teachings of the specification, I have made a transgenic tobacco plant that expressed an antisense construct comprising a nucleic acid molecule encoding a tomato RdRP. Similar to Xie, I found that transgenic

tobacco plants containing the tomato RdRP cDNA as an antisense construct exhibited drastically reduced RdRP activity after treatment with TMV and potato spindle tuber viroid (PSTVd) as shown by Northern analysis and expression of more severe symptoms upon TMV infection. Thus, the experiment demonstrates that the specification enables production of a transgenic plant comprising a heterologous antisense RdRP and that the resulting antisense construct resulted in reduced RdRP activity.

13. Furthermore, it was well known in the art at the time the invention was made that co-suppression could be used to reduce the expression of the homologous gene in plants (WO 90/12084). For this reason, I believe and reasonably expect that one of ordinary skill in the art could similarly use the nucleic acids of the invention to produce transgenic plants without undue experimentation and would reasonably expect the plants to exhibit reduced RdRP activity due to co-suppression.

14. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Michael Wassenegger

Signed this 07 day of May, 2003
at Munich, Germany